# Bacterial Abundance, Production and Community Composition in Thin Biological Layers

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#### LONG-TERM GOALS

My long term goal in this project is to determine the small-scale (centimeter-scale) distribution of heterotrophic bacterioplankton and their activities as they relate to the distribution of other biological components of the foodweb as well as the physical structure of the water column.

#### **OBJECTIVES**

The objective of this research is to (1) determine whether the distribution of heterotrophic bacteria in thin layers shows similar patterns to that of phytoplankton and (2) determine whether the activities of heterotrophic bacteria within the thin layers are significantly different than those in the surrounding water. (3) determine whether the composition of the community of bacteria associated with layers differ from the surrounding communities

### **APPROACH**

<u>Sample collection:</u> Water samples were collected using two separate techniques. With Dian Gifford (URI), a siphon-based profiling and collection system modified from Donaghay *et al.* (1992) was used to both characterize the water column and collect discrete water samples. Additionally, a free-fall profiling system (with Tim Cowles, OSU) was used to characterize the water column and collect discrete water samples. Specific depths were targeted for the collection of discrete water samples on the basis of beam attenuation (siphon sampler) or *in situ* fluorescence (free-falling profiler).

<u>Bacterial assays:</u> Bacterial abundance was determined using epifluorescnence microscopy after staining samples the DNA fluorochrome 4',6-diamidino-2-phenylindole (DAPI; Porter and Feig 1980). Bacterial growth rates were assayed using the <sup>3</sup>H-leucine incorporation method (Kirchman *et al.* 1985) as modified for small-scale samples (Smith and Azam 1992).

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#### WORK COMPLETED

I participated in the June 1998 Thin Layers field program conducted in East Sound, Orcas Island, WA. Centimeter-scale sampling of the water column during thin layer events was successfully conducted using both profilers on board the *R/V Henderson*. Bacterial abundance and growth was determined in discrete water samples collected through the water column. These data were analyzed with respect to chlorophyll concentrations.

#### RESULTS

Vertical profiles of bacterial growth rates exhibited similar trends to chlorophyll a profiles in the water column (Figure 1).

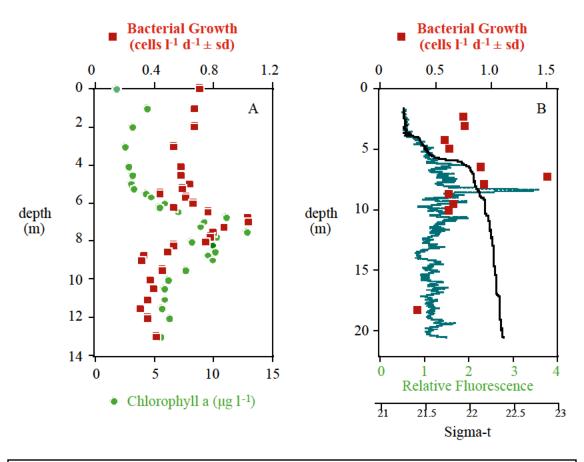


Figure 1. Two vertical profiles of bacterial growth in thin layers. A. Profile collected 20 June 1999 using the siphon system. B. Profile collected 22 June 1999 using the free-fall profiling system.

In the two profiles, bacterial growth rates increased ~3-fold within the thin layers of chlorophyll. This suggests that heterotrophic batcheria respond to the uniques conditions within the thin layers on time scales compatible with the formation and persistance of the

thin layers. To date, bacterial abundance and growth has been analyzed with chlorophyll concentrations exclusively. Analysis of these data with other parameters (DOC, nutrients *etc.*) is planned.

It is very likely that bacterial growth in the thin layers was stimulated by increases in dissolved organic matter (DOM) associated with the phytoplankton. Several mechanisms for the production of DOM from phytoplankton have been studied, these include direct exudation (Fogg, 1983), sloppy feeding (Lampert, 1978), viral lysis (Fuhrman, 1992) and enzymatic hydrolysis (Smith *et al.* 1995). While the shapes of the vertical profiles of bacterial growth and chlorophyll are very similar they are not identical. Bacterial growth rates above the chlorophyll peak (Figure 1A) are higher relative to the growth rates below the chlorophyll peak. Concurrent grazing experiments in this layer (D. Gifford, URI) indicate that chlorophyll was being grazed at a higher rate in and above the peak. This suggests that 'sloppy feeding' may have been a dominant mechanism in DOM production in this thin layer.

#### IMPACT/APPLICATIONS

The use of the small-scale method for measuring bacterial growth rates employed in this study worked extremely well. The replicate samples (three per depth) showed remarkably little variation (Figure 1). From a practical standpoint, implementation of this method allowed replicate sampling from the small initial sample volume (500 ml with the free-falling vertical profiler) while having enough water remaining to conduct other assays (nutriets, DOC *etc.*). More importantly, these data suggest that actual variability normally seen in these assays may be a function of the sampling method. This futher points to the need to sample on spatial scales that dominate the biological process of interest.

#### **TRANSITIONS**

The process of bacterial growth in the sea comes at the expense of dissolved organic matter. As DOM plays an important role in ocean optics, consideration of this critical component in controlling the dynamics of the DOM pool must be included in optical models. Concomitant with the consumption of DOM during bacterial growth is the conversion of DOM to the particulate phase (*i.e.* new bacterial cells). This repackaging of organic matter from the dissolved phase (DOM) into particulate phase (i.e.  $\sim 1~\mu m$  cells) should also be considered in the study of ocean optics.

#### RELATED PROJECTS

This work is done in collaboration with the following ONR Principal Investigators during the 1998 East Sound field program:

Dian Gifford (University of Rhode Island) - Intensive sampling with centimeter-scale resolution provided data sets that will allow us to study relationships among the microbial components in the thin layers. In addition, grazing experiments conducted by Dr. Gifford

may provide evidence for the mechanisms which enhance the bacterial growth rates within the layers.

Timothy Cowles' (Oregon State University) - Microbiological assays were conducted on samples from Dr. Cowles free-fall vertical profiler. Both continuous data collected by the profiler (fluorescence, density *etc.*) and data collected from the discrete water samples (nutrients, DOC *etc.*) will be used to analyze the distribution of bacteria and their activities.

Mary Jane Perry (University of Washington) - Bacterial growth rates assays were conducted in subsamples of water used by Dr. Perry for the P vs. E curves. These data will allow for direct comparison of contemporaneous organic matter production (*via* photosynthesis) and organic matter consumption (*via* bacterial growth).

Van Holiday, (Marconi Aerospace Defense Systems) - Dr. Holiday's real-time acoustical data facilitated our water sampling scheme by indicating the presence of thin layers. His data will allow the microbiological data from the discrete water samples to be interpreted in a broader context by using the extent and duration of the layers.

Percy Donaghay and Margaret McManus Dekshenieks (University of Rhode Island) - Water (and thin layer) movement within East Sound documented by Drs. Donaghay and Dekshenieks will also allow a broader interpretation of the microbiological data collected in the discrete water samples.

Jan Rines (University of Rhode Island) - The microbiological data will be studied with respect to the detailed knowledge of the makeup of the diatom community inhabiting the waters in East Sound during our field program that Dr. Rines collected.

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## **PUBLICATIONS**

Smith DC. 1999. Bacterial growth and abundance in biological thin layers. American Society of Limnology and Oceanography 1 - 5 Feb 1999:Santa Fe, NM. (Abstract)